MISSOURI DEPARTMENT OF NATURAL RESOURCES
AIR AND LAND PROTECTION DIVISION
ENVIRONMENTAL SERVICES PROGRAM
Standard Operating Procedures

SOP #: MDNR-WQMS-109 EFFECTIVE DATE: 08/05/05

SOP TITLE: Analysis of Escherichia coli and Total Coliforms Using the IDEXX Colilert and Quanti-Tray Test Method

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SUMMARY OF REVISIONS: Not applicable. This is a new standard operating procedure.

APPLICABILITY: The procedures outlined in this SOP apply to all ESP personnel

DISTRIBUTION: MDNR Intranet
ESP, SOP Coordinator

RECERTIFICATION RECORD:

Date Reviewed				
Initials				

1.0 SCOPE AND APPLICABILITY

This Standard Operating Procedure provides Environmental Services Program (ESP) staff with guidance on the detection and enumeration of Escherichia coli and total coliforms in water using the Colilert and Quanti-Tray system. The method can be applied to ambient fresh waters, drinking waters and wastewaters (see Colilert package insert). The Colilert method is not intended for use with marine waters.

2.0 PERSONNEL QUALIFICATIONS

Field personnel must have a working knowledge of field sample collection procedures. Staff shall have, at a minimum, attended the department-sponsored Inspection and Enforcement training, Basic Sampling Workshop, or received training from another MDNR employee knowledgeable on proper sample collection procedures (see MDNR-FSS-001).

3.0 HEALTH AND SAFETY

Field activities involving the collection of bacterial samples involve working in or around various waste streams and water bodies. Field personnel should protect themselves by wearing the appropriate level of personal protective equipment such as disposable gloves and waders. The analyst should also have knowledge of safety procedures required in a microbiology laboratory that prepares, analyzes and disposes of contaminated water.

Personnel should participate in medical monitoring in accordance with the MDNR medical monitoring policy. All field personnel who are routinely exposed to domestic and animal waste should be familiar with the Hepatitus A Prevention vaccine policy. Both policies can be reviewed on the MDNR's home page (or ESP intranet) by accessing the Health and Safety information page.

4.0 GENERAL OVERVIEW

4.1 The Colilert method is based on Defined Substrate Technology. The product utilizes nutrient indicators that produce color and/or fluoresce when metabolized by total coliforms and E. coli. After the reagent is added to the sample and incubated, it can detect these bacteria at 1 MPN/100mL within 24 hours with as many as 2 million heterotrophic bacteria /100mL present.

4.2 For this method, coliform bacteria are those bacteria which produce a yellow color, and for E. coli, also fluoresce under a 6-watt, 365 nm UV light after incubation at 35^0 C ± 0.5^0 C for 24 hours.

4.3 The method may be used as a Presence/Absence test or, more commonly in the ESP, a quantification test using the Quanti-Tray system.

4.4 Since a wide range of bacterial levels may be found in surface waters and wastewaters, dilutions can be used with this method for detecting and enumerating the actual level. The minimum, non-zero number of bacterial counts detectable with this method is a function of the dilution scheme used when processing the sample.

5.0 EQUIPMENT AND SUPPLIES

5.1 Sterile, bacteriological, to deliver (T.D.), pipettes (plastic or glass) of appropriate volume

5.2 Sterile vessels, glass or plastic (fluorescence free), 100-200mL volume

5.3 Incubator maintained at 35^0 C ± 0.5^0 C

5.4 A 6-watt, 365-nm, UV light

5.5 Quanti-Trays of either the 51 or 97 well variety

5.6 Quanti-Tray sealer (115 V)

5.7 Colilert comparator(s)

6.0 REAGENTS

6.1 Sterile deionized or distilled water

6.2 Sodium thiosulfate for dechlorinating water samples. Sterile sample collection containers may be purchased with sodium thiosulfate added.

6.3 Colilert reagent

7.0 SAMPLE COLLECTION AND PRESERVATION

7.1 A grab sample containing a minimum of 100 mL of sample should be collected in a sterile 120 mL plastic container. A sterile Whirl-Pak bag may be substituted but should contain a minimum of 100 mL of sample. If the water to be analyzed has been disinfected with chlorine, the collection vessel should either contain a small amount of sodium thiosulfate prior to sampling or a small amount added to the container after collection to neutralize any remaining chlorine. The sample container should be placed on ice in a cooler after collection. Refer to MDNR-FSS-

005 for the proper collection of grab samples and general sampling considerations.

Each sample container will be labeled with a sample number and the date and time of collection. This information will be documented on the Chain-of-Custody record (refer to MDNR-FSS-002 *Field Sheet and Chain-of-Custody Record* and MDNR-FSS-003 *Sample Numbering and Labeling*).

7.2 When collecting grab samples using a sterile plastic bottle, samples should be collected by holding the sample bottle near its base and plunging it (neck down) below the surface of the water. The bottle should be turned until the neck of the bottle points slightly upward. If a water current is present, the mouth of the bottle should be directed into the current. The bottle should be filled to the 100 mL mark. If possible, the sample bottle should be capped while the bottle is still submerged below the surface of the water. When sampling waters that have been chlorinated or suspected to contain chlorine, sodium thiosulfate must be used to neutralize its effects. The chemical can be added immediately after collection or using pre-preserved sterile containers. If using pre-preserved sample containers, it is important that care be taken when filling the containers so as not to lose the sodium thiosulfate from the container. This may be accomplished by holding the bottle by the neck and gently lowering it below the surface of the water while slightly tipping the mouth upstream.

7.3 When collecting grab samples using a Whirl-Pak bag a Whirl-Pak water scoop should be used. The security tab should be removed, the tabs pulled out and the wire ends secured to the water scoop prior to submerging the Whirl-Pak bag. Submerge the bag under the water surface and slowly move the bag into the current (if present). To aid in filling, the bottom of the bag may be held as the bag is moved into the current. The bag should be filled to at least the 100 mL line. If possible, the bag should be closed while it is submerged below the surface of the water. To close the bag, remove from the water scoop and grip the wire ends (one in each hand). Carefully twirl the bag approximately three revolutions then turn the wire ends inward to the opposite face of the fold and twist together. It will be necessary to transfer a known volume of sample to a sterile 120 mL vessel and bringing it up to 100 mL mark prior to adding the Colilert reagent

7.4 Sample analysis should be conducted as soon as possible after collection. The holding time requirement will depend on the Quality Assurance Project Plan being implemented. Typical holding times are 6 hours for non-potable water and 24 hours for drinking water compliance. Additional information regarding handling procedures can be found in MDNR-FSS-018 *Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab*.

8.0 QUALITY CONTROL

8.1 Each lot of Colilert reagent should be checked by running both a negative control (reagent +100 mL sterile water) and a positive control (typically Jefferson City Wastewater Treatment Facility influent + reagent). The expected result should yield a colorless sample for the negative control and a yellow fluorescent sample for the positive sample. **Note: For an accurate comparison of positive results, a color comparator supplied by IDEXX can be located in a cabinet in the WQMS laboratory.**

8.2 Analytical bench sheets must include the lot number and expiration date of the reagent, specific Quanti-Tray used, and sterile dilution water (if applicable).

8.3 Counts of positive wells should be verified by either the analyst counting twice or by having another analyst make a determination. Positive wells should exhibit both the color and intensity of the comparator.

9.0 CALIBRATION AND STANDARDIZATION

9.1 Incubator temperature should be checked each day of analysis to ensure compliance with the $35^{o}C \pm 0.5^{o}C$ requirement.

9.2 Incubator thermometers should be checked annually against an NIST certified thermometer.

10.0 ANALYTICAL PROCEDURES

The IDEXX Colilert method may be used as a simple Presence/Absence test or results can be quantified by using either the 51 or 97 well Quanti-Tray. Under normal ambient conditions the 51 well tray is suitable for most surface waters. In situations of known point source influence or storm water, the 97 well tray should be used. Below are descriptions of both procedures.

10.1 Presence/Absence

10.1.1 Inspect the sample to be analyzed to ensure that the appropriate sterile container was used. Make sure the sample contents reach the 100 mL line on the container. Sample may be removed to reach the 100 mL level. For those samples under 100 mL the analyst may either; 1) add non-buffered distilled or deionized sterilized water to bring up to 100 mL or, 2) if only slightly less than 100 mL (10 mL or less) analyze as is. In either case, document on the bench sheet the steps taken.

10.1.2 Carefully remove one packet of Colilert reagent from strip taking care not to accidentally open adjacent packet.

10.1.3 Tap the packet of reagent to ensure that all powder is in the bottom of the packet.

10.1.4 Open the packet by snapping the top along the scored line.

10.1.5 Add the packet of reagent to the 100 mL sample.

10.1.6 Cap and seal the container making sure as to not contaminate the contents.

10.1.7 Shake the sample vigorously until the reagent is dissolved.

10.1.8 Incubate the sample at 35^0 C ± 0.5^0 C for 24 hours.

10.1.9 After 24 hours the results may be interpreted as follows;

- If no yellow color is observed the test is negative for the presence of total coliforms.

- If the sample has a yellow color equal to or greater than the comparator, the test is positive for total coliforms. If the color is not uniform, mix the contents by inverting the container several times then recheck.

- If the sample is yellow but lighter in color than the comparator, it may be incubated an additional 4 hours (but no more than 28 hours total). If the sample is positive for total coliforms the color will intensify. If the color does not intensify, the sample is negative.

- A sample which exhibits a yellow color may be checked for fluorescence by placing in the viewing cabinet containing a 6-watt, 365-nm UV light source. If a viewing cabinet is not available, a 6-watt, 365-nm UV light source may be used by holding within 5 inches of the sample in a dark environment. **Caution: Do not look directly at the UV light. Serious eye damage could result.** If the fluorescence is equal to or greater the fluorescence of the comparator, the sample is positive for E. coli.

10.2 Quantification

10.2.1 Turn the Quanti-Tray Sealer on to allow the instrument to heat up. Allow several minutes for the sealer to reach proper operating temperature. An orange light indicates the power is on. When the instrument has warmed to the required temperature a green light will appear immediately below.

10.2.2 Most Probable Number (MPN) counts can be obtained by using the Colilert reagent and the IDEXX Quanti-Tray system. Counts from <1 through 200.5 (per 100 mL) can be obtained with the 51-well tray while counts up to 2419.6 may be achieved with the 97-well tray (see Quanti-Tray package insert). Higher counts can be obtained by making the appropriate dilution.

10.2.3 A dilution can be made if a sample is suspected to contain high numbers of either total coliforms or E. coli. and a more accurate appraisal of the MPN is desired.

10.2.4 Use only sterile distilled or deionized water in making dilutions. Buffered dilution water is not necessary as the Colilert reagent is already buffered. The reagent must be added to a 100 mL volume (sample + dilution water) before addition to the selected Quanti-Tray.

10.2.5 Shake the sample container vigorously to help dissolve the powdered reagent.

10.2.6 Allow the sample to set if excessive foaming is present.

10.2.7 Mark the back of the selected Quanti-Tray with the corresponding sample number and enter all the information (such as date/time collected, dilution factor, incubation temperature and start time) on the bench sheet (see attached).

10.2.8 Squeeze the top of the selected Quanti-Tray to open and pour the contents (sample + reagent) from the container into the Quanti-Tray while avoiding contact with the foil tab at the top.

10.2.9 Set the Quanti-Tray into the appropriate rubber insert (51 or 97 well) while keeping the tray in an upright position and insert into the sealer. **Note: If insert becomes stuck you may use the reverse button located above the tray holder in the middle of the sealer unit.**

10.2.10 Remove the sealed tray from the sealer and incubate at 35^0 C ± 0.5^0 C for 24 hours.

11.0 CALCULATIONS AND DATA ENTRY

11.1 Quanti-Tray

11.1.1 Follow the same general interpretation results from Section 10.1.9 to count the number of positive wells. Refer to the appropriate MPN table (51 well or 97 well) provided with the Quanti-Tray system to determine the MPN of total coliforms (yellow wells) and E. coli (yellow/fluorescent wells) in the sample. **Note: The color and fluorescent intensity of positive wells may vary and should be examined in relation to the comparator.**

11.1.2 Record the results on the bench sheet as the Most Probable Number/100 mL. If any dilutions were made, multiply the MPN/mL by the dilution factor to obtain the final MPN/100mL value.

11.1.3 When evaluating results from multiple dilutions, the least diluted tray exhibiting a mix of positive wells and negative wells closest to 80% positive and 20% negative should be the MPN reported.

11.1.4 The analyst should then enter the results from the bench sheet into the Laboratory Information Management System (LIMS). The data is then validated by the unit supervisor and approved by the manager of the section. The completed bench sheet will be maintained by the WQMS.

11.2 Presence/Absence

11.2.1 At the present time, the ESP does not use the presence/absence test on any samples and therefore, no documentation is required.

11.2.2 At such time the test is warranted, modifications to the bench sheet and LIMS will be made to accommodate the needed information.

12.0 WASTE MANAGEMENT AND POLLUTION PREVENTION

12.1 The reagent and solutions generated by this method pose little threat to the environment when managed properly. For further information see the applicable Material Safety Data Sheet (MSDS).

12.2 Sample containers, Quanti-Trays, solutions, and reagents remaining after analysis shall be disposed of according to accepted laboratory practices.

Remaining sample and solutions containing sample and reagent may be disposed of through the sanitary sewer. Quanti-Trays containing sample and reagent should be treated as a potential bio-hazard and either sterilized before disposal or incinerated.

13.0 REFERENCES

Colilert Package Insert from IDEXX

IDEXX Colilert Test Method for the Simultaneous Detection of Total Coliforms and E. coli in Water, Draft Document, June 2003

MDNR-FSS-001 *Required/Recommended Containers, Volumes, Preservatives, Holding Times, and Special Sampling Considerations.*

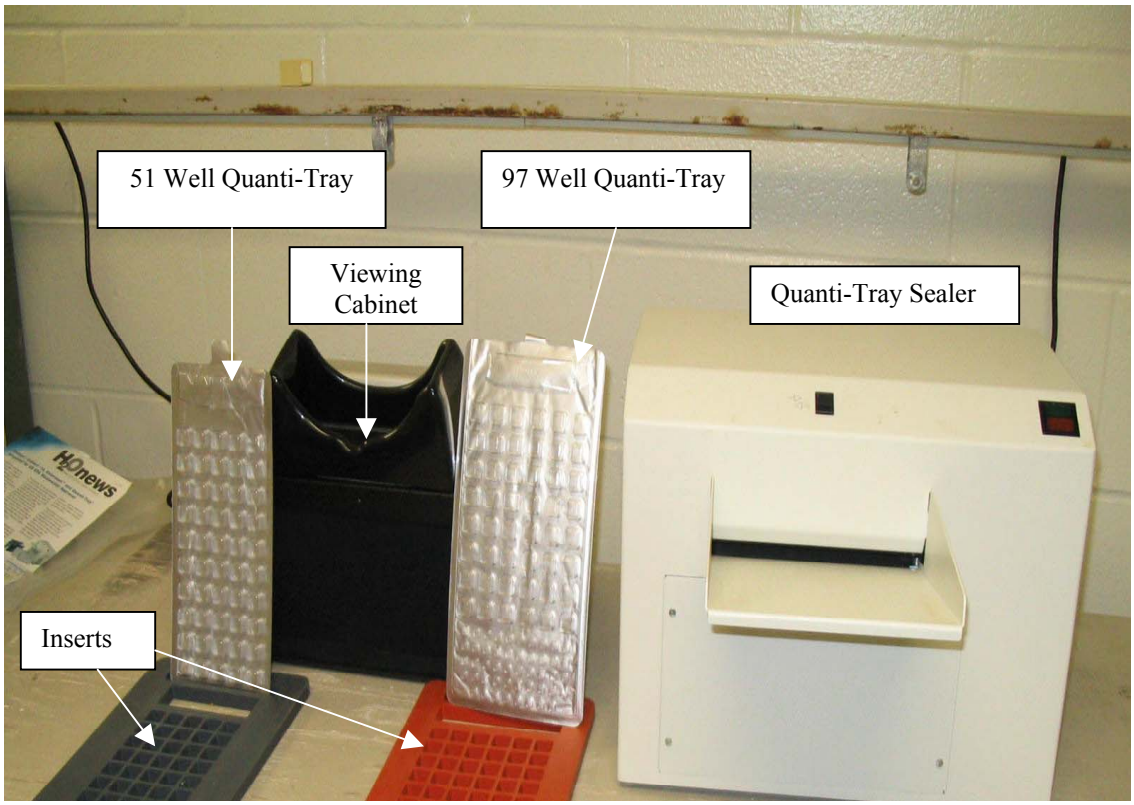
MDNR-FSS-003 *Sample Numbering and Labeling*

MDNR-FSS-005 *General Sampling Considerations Including the Collection of Grab, Composite, and Modified Composite Samples from Streams and Wastewater Flows.*

MDNR-FSS-018 *Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab.*

Quanti-Tray Package Insert from IDEXX

IDEXX QUANTI-TRAY SYSTEM



LDPR Code_____

Missouri Department of Natural Resources
E. Coli Analysis Bench Sheet
(For use with the Colilert® test method SM9223)

Sample Information			Volume of Raw Sample Analyzed *(mL)*	Quanti-Tray™ Results Using Well *(check one)*		Total Coliform					E. coli				
Sample Number	Collected					# of Positive Wells				MPN/100 mL	# of Positive Wells				MPN/100 mL
	Date	Time		51	97	Large	Small	MPN	DF		Large	Small	MPN	DF	

Verified Count

MPN = Most Probable Number
DF = Dilution Factor

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Missouri Department of Natural Resources
E.Coli Analysis Bench Sheet
(For use with the Colilert® test method SM9223)

	LOT NUMBER	EXPIRATION DATE
REAGENT (IDEXX)		
DILUTION WATER (NON-BUFFERED)		
QUANTI-TRAY ™ 51 WELL		
QUANTI-TRAY ™ 97 WELL		

	INCUBATION START	INCUBATION END
DATE		
TIME		
TEMPERATURE (°C)		
ANALYST		

VERIFICATION METHOD (CHECK ONE):	DATA ENTRY INTO LIMS	
☐ WELLS COUNTED TWICE BY:	DATA ENTERED BY:	DATE:
☐ WELLS COUNTS VERIFIED BY:	DATA VALIDATED BY:	DATE:
	DATA APPROVED BY:	DATE:

COMMENTS:

MPN = Most Probable Number
DF = Dilution Factor

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